

CLAIMS

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What is claimed is:

- SUB D1*
1. A method of amplifying complementary first and second nucleic acid sequences each of which has a binding region at its 3' end, the method comprising treating the separated single stranded sequences with
 - (a) first and second primers each capable of hybridising to the 3'-binding regions of the first and second strands respectively and each including remote from its 5'-end a digestion resistant region which, with the primer hybridized to the complementary 3'-binding region, allows only partial digestion of the primer by the enzyme (d) having 5'-double strand specific exonuclease activity,
 - (b) third and fourth primers each having a degree of sequence homology with the partially digestible regions of the first and second primers respectively whereby the third and fourth primers are capable of hybridising to the 3'-binding regions of the first and second strands respectively,
 - (c) an enzyme having strand displacing polymerase activity,
 - (d) an enzyme having 5' double stranded specific exonuclease activity, said enzyme (d) possibly being provided by enzyme (c) in the case where the latter also has the required exonuclease activity, and
 - (e) nucleoside triphosphates,

under conditions permitting hybridisation, exonuclease digestion and strand displacement polymerisation thereby producing an amplified amount of the first and second strands.

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2. A method as claimed in claim 1 wherein the double stranded nucleic acid molecule is generated *in situ* from a single stranded nucleic acid molecule.

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3. A method as claimed in claim 1 or 2 wherein the digestion resistant region is provided by modified nucleotides or ribonucleotides.

4. A method as claimed in claim 3 wherein the modified nucleotides provide phosphorothiate linkages which provide the resistance to digestion by the exonuclease.

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5. A method as claimed in any one of claims 1 to 4 wherein the first and second primers each comprise 30 to 60 bases.

6. A method as claimed in claim 5 wherein the digestion resistant region is provided 15 to 25 bases from the 5' end of the first and second primers.

7. A method as claimed in any one of claims 1 to 6 wherein the third primer is of a sequence corresponding to at least a portion of the sequence in the first primer on the 5' side of the digestion resistant region of that primer.

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8. A method as claimed in any one of claims 1 to 7 wherein the fourth primer is of a sequence corresponding to at least a portion of the sequence in the second primer on the 5' side of the digestion resistant region of that primer.

9. A method as claimed in any one of claims 1 to 8 wherein the third and fourth primers comprise 12 to 30 bases.

10. A method as claimed in any one of claims 1 to 9 wherein the 5' double strand specific exonuclease is T7 Gene 6 exonuclease.

11. A method as claimed in any one of claims 1 to 10 wherein the strand displacing DNA polymerase is at least one of, 9°N polymerase, Klenow (exo⁻) polymerase, *Bst* polymerase, Vent (exo⁻) polymerase, or Deep Vent (exo⁻) polymerase, *Pfu* (exo⁻) polymerase, *Tth* polymerase, *Tfl* polymerase, *Taq* polymerase or *Bca* (exo⁻) polymerase.
12. A method as claimed in any one of claims 1 to 11 wherein the steps of exonuclease digestion and strand displacing polymerisation are effectively separated by performing the two reactions separately by removal of enzyme between steps, or, under conditions which favour the action of one or other enzyme.
13. A method as claimed in any one of claim 1 to 12 effected isothermally.
14. A method as claimed in anyone of claims 1 to 13 wherein the digestible regions of the first and second primers are of identical sequence and the third and fourth primers are identical to these sequences.
15. A method as claimed in anyone of claims 1 to 14 wherein the 5'-ends of the first, second, third and fourth primers have a partial degree of resistance to digestion.
16. A method as claimed in any one of claims 1 to 15 wherein the amplification occurs in the presence of further primers specific to other target sequences (multiplex amplification) or to all or some of the same target sequence (nested amplification).
17. A method as claimed in any one of claims 1 to 16 wherein at least a portion of at least one of the nucleoside triphosphates provided as (e) of claim 1 is/are a modified such that when it is incorporated in a growing nucleic acid chain it is resistant to digestion by the exonuclease.

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18. A method as claimed in any one of claims 1 to 17 wherein the nucleic acid is DNA.

19. A method of amplifying complementary first and second nucleic acid sequences each of which has a binding region at its 3' end, the method comprising the steps of

- (i) forming a reaction mixture comprised of the separated single strands together with

(a) first and second primers each capable of hybridising to the 3'-binding regions of the first and second strands respectively and each including remote from its 5'-end a digestion resistant region which, with the primer hybridised to the complementary 3'-binding region, allows only partial digestion of the primer by the enzyme (d) having 5'-double strand specific exonuclease activity,

(b) third and fourth primers each having a degree of sequence homology with the partially digestible regions of the first and second primers respectively whereby the third and fourth primers are capable of hybridising to the 3'-binding regions of the first and second strands respectively,

(c) an enzyme having strand displacing polymerase activity,

(d) an enzyme having 5' double stranded specific exonuclease activity, said enzyme (d) possibly being provided by enzyme (c) in the case where the latter also has the required exonuclease activity, and

(c) nucleoside triphosphates.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

fecting a reaction under conditions of strand displacement polymerization of the first and second strands.

Sub B2

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under conditions permitting hybridisation, exonuclease digestion and strand displacement polymerisation thereby producing an amplified amount of the first and second strands.

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